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THYROID AND GONAD AS FACTORS IN THE PRODUCTION OF PLUMAGE MELANINS IN THE DOMESTIC FOWL.[1]

BENJAMIN HORNING AND HARRY BEAL TORREY.

I.

In a recent paper, Zavadovsky (1925, 1) has described the production of white feathers on pigmented fowls following thyroid feeding. The dosage was excessive. Some of the birds succumbed; all were highly intoxicated. The survivors soon moulted. Back and wing feathers fell abundantly in the course of ten days, and were succeeded by feathers that were partly or wholly white. Flesh feeding did not produce these effects, nor adrenal gland. The author concluded that "the thyroid plays a specific rôle in the regulation of the growth and moulting of feathers and in their pigmentation."

In the next issue of the same publication, Zavadovsky (1925, 2) described an experiment in which a mongrel black hen under whose skin five dog thyroids had been grafted, developed tufts of white feathers at the seat of the graft. He also observed that "the new plumage which makes its appearance after the experimental moulting is apparently much softer than the old ordinary plumage."

Since 1921, we have had some hundreds of thyroid-fed fowls under observation, and have repeatedly noticed in their plumage occasional feathers with defects in pigmentation similar to those described by Zavadovsky. These have not been regarded as satisfactory evidence of a specific rôle of the thyroid in feather pigmentation. While they could be induced by thyroid feeding,

[1] The work on which this paper is based was done in the Zoölogical Laboratory, University of Oregon.

this was especially true when the dosage was sufficiently large to induce structural defects in the vane as well. Similar defects could be produced by inanition alone. Furthermore, defective pigmentation appeared in birds that were not on a thyroid diet and showed no general signs of hyperthyroidism. When such defects in pigmentation appeared in birds receiving the dosage of thyroid commonly employed by us, namely, 1 gram of Armour and Company's desiccated thyroid to 5,000 grams of body weight, the non-pigmented areas, when they occurred at all, were usually limited to the tips of the feathers involved. This was strictly true for the feathers of the trunk. In wing quills, the extent of the defect was sometimes larger.

With these facts in mind, we are disposed to refer the striking change in plumage recorded by Zavadovsky to a metabolic disturbance induced by an excessive, essentially toxic dosage of thyroid rather than to a specific action of the latter on the pigment-forming mechanism. The results of the graft mentioned in his second paper are not unfavorable to this view. We have not seen the extraordinary casting of feathers in adult birds which we suspect of being a further sequel of toxic feeding. But the moulting process, like the pigment-forming mechanism, is subject to modification under the influence of non-toxic doses of thyroid. This has been referred to in a preliminary note (Horning and Torrey, 1923, 2) and considered more fully in a recent paper (1925, 1). In the latter, two kinds of results of thyroid feeding were discriminated, the one referable to nutritive (associated with toxic), the other to non-nutritive factors. And shortly after (1925, 2), changes in feather structure were described which were not referable to changes in nutrition or to intoxication.

In the present paper, another non-toxic, non-nutritive effect of thyroid feeding will be described, namely, an increased pigmentation. Whereas Zavadovsky's birds *blanched* and not infrequently died under the massive doses pressed upon them, our birds, with a daily ration of thyroid that permitted them to maintain their health, lay viable eggs and rear normal offspring, grew *darker*.

II.

This darkening of the plumage, to which we originally called attention in a preliminary note (1923, 1) was also observed by Cole and Reid (1924). The plate accompanying their paper illustrates very well certain typical color changes produced in the feathers of Brown Leghorns by thyroid feeding. With a view to repeating our observations (1922) on the appearance of plumage of the female type on young Rhode Island Red males as a result of thyroid feeding, these authors found in thyroid fed Brown Leghorn cockerels that had assumed adult plumage changes in feather form and structure that are probably typical responses of poultry in general, but which Crew and Huxley (1923), on a similar errand, failed to note.

What is true of Brown Leghorns in this connection is also true of Campines, Barred Plymouth Rocks and Rhode Island Reds. White Leghorns show similar changes in feather form and structure, though not in color. This exception is likely to prove the rule among other dominant whites as well. There is a definite correlation between structure and color in these experiments, however, which brings both pigmented and non-pigmented races into a single category.

While these different races responded thus similarly to thyroid feeding, they did not do so with equal intensity. Of our pigmented races, the Brown Leghorn was most affected, the Rhode Island Red least. These differences in reaction associated with differences of race, however, were not conspicuous, nor are they so interesting, as those associated with differences in sex.

The results of thyroid feeding are peculiarly conspicuous in Brown Leghorn males, especially in neck hackles, shoulders, backs and saddles, all feathers that are brilliantly colored in shades of red and show marked sexual dimorphism. In the male, these feathers are distinguished by a lacy border of naked barbs (Fig. 1). On a thyroid diet, the latter tend to clothe themselves to their tips with extensions of the normal two rows of barbules (Fig. 2). As the barbules thus extend toward the edge of the feather, so also does the melanin pigment; for, as a rule, this pigment is carried by the barbules and limited by their distribution. As a result of thyroid feeding, the zone of barbules

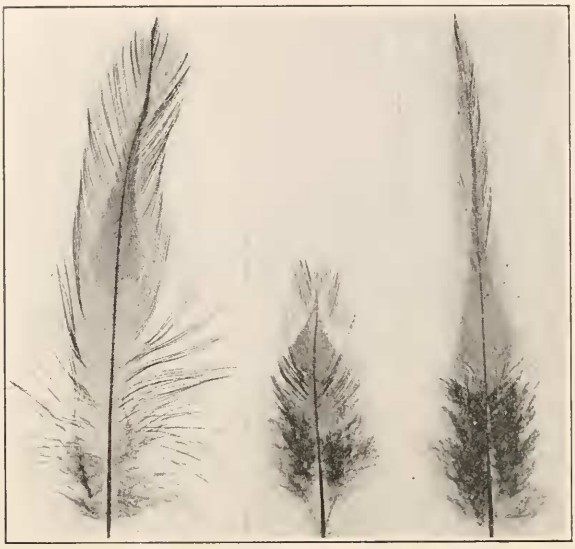

FIG. 1. Representative feathers from a normal adult B. L. male. From left to right they are: hackle, shoulder, saddle.

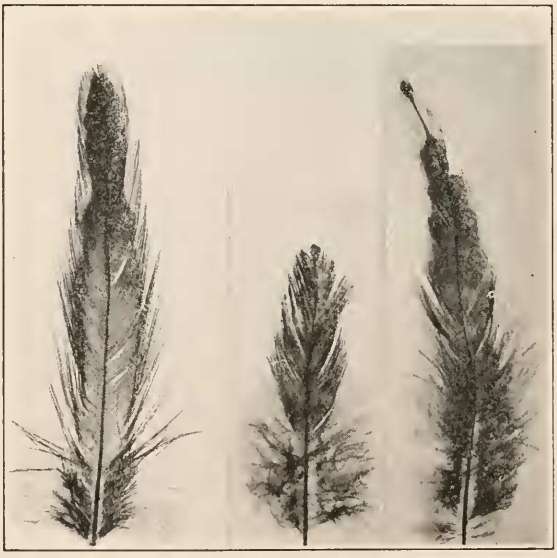

FIG. 2. Representative feathers from a thyroid fed adult B. L. male. From left to right they are: hackle, shoulder, saddle.

may extend beyond the zone of melanin pigmentation; but the reverse does not occur. In control birds, the reverse *does* occur. This is especially clear in the shoulder feathers of Barred Plymouth Rocks, in which dark bands pass distally across the naked barbs (Fig. 3).

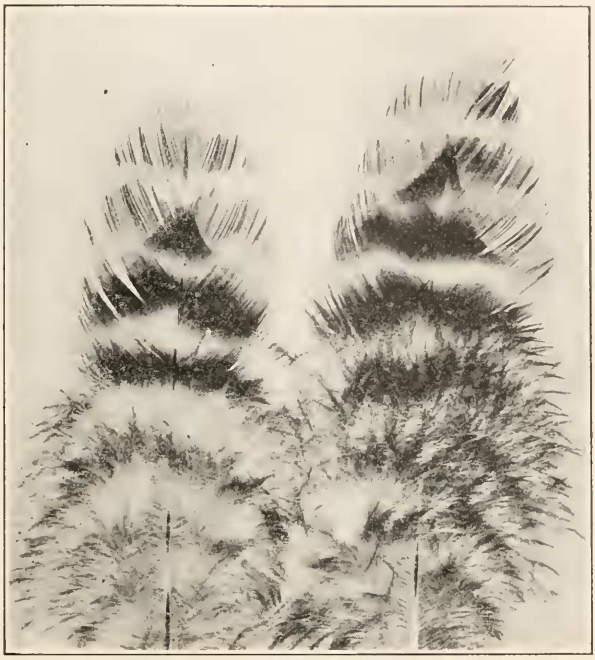

FIG. 3. Representative shoulder feathers from an adult Barred P. R. male, showing barring across naked barbs.

This tendency of barbules and melanin pigmentation to extend together over the feathers of thyroid fed birds is especially marked by reason of the fact that the zone of barbules in male birds possesses a typically notched or wavy contour with which the outer edge of melanin pigment distribution perfectly coincides. It is difficult to avoid the conclusion that the effective stimuli for barbule and for melanin formation in these feathers are one and the same. There is reason, however, for believing that this is not an adequate summary of the situation.

Everything that has been said about the male bird applies as well to the capon. In the latter, such decorative feathers

as the hackles, saddles and sickles are longer and possibly more highly colored. The plumage is more luxuriant, *more masculine.* That it becomes more deeply pigmented with melanins as a result of thyroid feeding, however, does not appear from our observations.

In sharp contrast with the effect of thyroid feeding on the coloring of the male bird is its effect on the normal female. Our evidence on this point has been obtained first, from females that had received a daily ration of thyroid approximately 1 : 5000 body weight for at least a year, beginning with the second to fourth week after hatching; second, from females approximately two years old that had been plucked in hackle and saddle regions to stimulate the eruption of new feathers and then placed on the same ration of thyroid as the others.

In the first group, thyroid feeding produced no departure in coloration from the controls. One conspicuous instance of this absence of thyroid effect was supplied by three hens whose parents as well as themselves had been on a thyroid ration practically all their lives. When a year old, they were not distinguishable from their controls.

In the second group were five hens. In the new feathers that appeared on the denuded areas during the period of thyroid feeding there was little or none of the brown mottling characteristic of the controls, and a correspondingly wider distribution of dark pigmentation, less intense than in corresponding feathers of thyroid males, but present in all the experimental birds.

If these observations accurately represent typical conditions, it may be said that the plumage of Brown Leghorn females darkens under the influence of thyroid feeding, as indicated, but not to the same degree as in males of the same age, and apparently not at all in birds in their first year.

A comparable difference in the effect of thyroid feeding on the two sexes has also been observed among Barred Plymouth Rocks. It is the male, rather than the female, whose plumage darkens in the course of the early moults.

These facts suggest a possible inhibitory influence of the ovary in this connection, a view which receives strong support from the following case.

FIG. 4. Thyroid fed B. L. female from which the ovaries have been removed.

FIG. 5. **Representative hackles** from the bird shown in Fig. 4, arranged from below upward.

A Rose Comb Brown Leghorn female, hatched April 9, 1923, was ovariotomized May 6, 1923. Thyroid feeding was begun at once. Fig. 4 is a photograph of this bird almost two years

FIG. 6. Representative saddle feathers from three adult B. L. females which are, from left to right; the bird shown in Fig. 4, a normal thyroid fed female, and a control.

later, just before she was killed on March 28, 1925. The comb and wattles are large and well formed. The spurs are 2 cm. long. Sickles are present among the feathers of the tail. These are all male characters, assumed as a consequence of ovariotomy. The body conformation is of the female type, as is, in general, the plumage. One exception to this statement has already been noted, namely, the presence of tail sickles. Another is to be found in the neck hackles which, though not well shown in the photograph, approximate in form and structure and color the hackles of thyroid fed male birds. In general, the plumage is duskier than in unaltered females. This is especially true for hackles and saddles. The former may have no lacing, or they may be laced intermittently in the fashion characteristic of thyroid fed males. In neither case, however, do traces of the mottling remain that is so conspicuous in the female hackle. The saddles, which are female in form and structure, show traces of mottling but along with the hackles show an intensity of melanin pigmentation that surpasses the results, mentioned above, on unaltered thyroid fed females.

In post mortem examination of this bird, no trace of a definitive ovary was seen. At the site of the latter, however, were certain small fleshy nodules which revealed, on sectioning, masses of tubules resembling testicular tubules in various stages of development from sex cords, though lacking in definitive sex cells. We have assumed no connection between this tissue and the changes in pigmentation before us. Its significance appears to lie in another direction; and its further consideration will be deferred to another occasion.

While barbule formation and melanin production are both stimulated by thyroid feeding in the male, the presence of the ovary in the thyroid fed female interferes with the second without interfering with the first. Indeed, barbule formation reaches its fullest expression in the female. Lacing, though present in the hackles of the female to a limited extent, is an elaborate and striking feature of those feathers in the male that are especially associated with sex.

The evidence is obviously not sufficient to warrant final conclusions regarding the inhibitory effect of the ovary on pigment formation stimulated by thyroid feeding. It is clear, however, that males and females respond differently to thyroid feeding. And that this difference is due in some measure to the influence of the ovary seems equally true. As for the measure of its influence and the nature of the mechanism by which it is achieved, the matter is not so simple and must await further investigation.

III.

Two classes of feather pigments have been distinguished: *lipochromes*, which are soluble in alcohol and ether and are red, rose brown, yellow and scarlet, and *melanins*, which range from sepia to black. In our experiments, the extension of the latter is correlated with the disappearance of the former. Cole and Reid mention "an evident action toward the reduction of red pigment." We have no evidence of an actual replacement of the one by the other, either by substitution or transformation. It is quite consistent with our observations to assume for the present that the former are merely concealed as the latter advance.

The melanins appear in definite chromatophores, being formed *in situ*. The chromatophores are found in the epidermis of the rachis and barbs. At first they are lacking in pigment which is gradually laid down within them in the form of granules. The pigment of the barbule cells is obtained by a direct transfer from the chromatophores which send out amœboid processes as the barbules develop, these processes meeting and fusing with the barbule cells as though guided by a tropistic factor. Readers who are interested in the details of this remarkable mechanism are referred to the papers of Strong (1902) and Lloyd Jones (1915).

The expansion of melanins in the feathers of thyroid fed birds is conceivably dependent, then, on several factors: an increased amount of melanin pigment; an expansion of individual melanophores, with or without added pigment; an increase in number of melanophores; a migration of melanophores. Considering the large areas ordinarily free of melanins that darken under thyroid feeding, it is difficult to avoid the conclusion that the total amount of melanin is increased. The extension of individual melanophores over very much smaller areas is certainly a fact. Whether or not the melanophores multiply we have not determined. The number of *functional* melanophores assuredly increases. We have no evidence of the migration of melanophores over the long distances required by the observed facts, and it appears to be highly improbable.

The darkening of feathers as a result of thyroid feeding is probably due chiefly, then, to an increase in melanin pigment and an increase in the number of melanophores functioning with or without an increase in the number of cells themselves. Thyroid feeding is thus conceived to promote pigment formation, directly or indirectly, and possibly cell division as well. These are two sufficiently diverse functions, both, in our opinion, indirect. Whether the melanophores do or do not multiply under thyroid influence, there is no doubt that other cells do, namely, the cells of the barbules whose number and extent is so markedly increased. But it is not clear at present in either case through what channels the thyroid acts to augment pigmentation or cell proliferation. Evidence has been given elsewhere (Torrey,

Riddle and Brodie, '25) for the view that thyroxin depresses the division rate when acting directly on Paramecium.

Summary.

The results of thyroid feeding on five breeds of domestic fowl are recorded. They vary with dosage and with sex. When the daily ration is compatible with good health, the males of pigmented breeds, both normal and castrated, tend to *darken* conspicuously owing chiefly to an increased production of plumage melanins. Normal females are affected, if at all, to a much slighter degree. Castrated females, however, resemble males in this respect as in others. *An antagonism thus appears between ovary and thyroid* as indicated by their relation to the formation of plumage melanins. The *blanching* of plumage observed after excessive doses of thyroid is attributed to a toxic effect, non-specific in character. A correlation between the effect of thyroid feeding on melanin production and feather structure is pointed out. White Leghorns show the latter but not the former, being dominant whites.

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